The use of small field of view 3T MRI for identification of articular cartilage defects in the canine stifle: An ex vivo cadaveric study

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Abstract: Noninvasive identification of canine articular cartilage injuries is challenging. The objective of this prospective, cadaveric, diagnostic accuracy study was to determine if small field-of-view, three tesla magnetic resonance imaging (MRI) was an accurate method for identifying experimentally induced cartilage defects in canine stifle joints. Forty-two canine cadaveric stifles (n = 6/group) were treated with sham control, 0.5, 1.0, or 3.0 mm deep defects in the medial or lateral femoral condyle. Proton density-weighted, T1-weighted, fast-low angle shot, and T2 maps were generated in dorsal and sagittal planes. Defect location and size were independently determined by two evaluators and compared to histologic measurements. Accuracy of MRI was determined using concordance correlation coefficients. Defects were identified correctly in 98.8% (Evaluator 1) and 98.2% (Evaluator 2) of joints. Concordance correlation coefficients between MRI and histopathology were greater for defect depth (Evaluator 1: 0.68-0.84; Evaluator 2: 0.76-0.83) compared to width (Evaluator 1: 0.30-0.54; Evaluator 2: 0.48-0.68). However, MRI overestimated defect depth (histopathology: 1.65 ± 0.94 mm; Evaluator 1, range of means: 2.07-2.38 mm; Evaluator 2, range of means: 2-2.2 mm) and width (histopathology: 6.98 ± 1.32 mm; Evaluator 1, range of means: 8.33-8.8 mm; Evaluator 2, range of means: 6.64-7.16 mm). Using the paired t-test, the mean T2 relaxation time of cartilage defects was significantly greater than the mean T2 relaxation time of adjacent normal cartilage for both evaluators ($P < 0.0001$). Findings indicated that MRI is an accurate method for identifying cartilage defects in the cadaveric canine stifle. Additional studies are needed to determine the in vivo accuracy of this method.

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Title: The Use Of Small Field Of View 3T MRI For Identification Of Articular Cartilage Defects In The Canine Stifle: An Ex Vivo Cadaveric Study

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Abstract

The objective of this study was to determine if small field of view 3.0 tesla (3T) MRI was capable of identifying experimentally induced cartilage defects in canine stifles. Forty-two canine cadaveric stifles (n=6/group) were treated with sham control, 0.5, 1.0, or 3.0 mm deep defects in the medial or lateral femoral condyle. PD-weighted, T1-weighted, FLASH, and T2 maps were generated in dorsal and sagittal planes. Defect location and size were independently determined by two evaluators and compared to histologic measurements. Accuracy of MRI was determined using concordance correlation coefficients. Defects were identified correctly in 98.8% (Evaluator 1) and 98.2% (Evaluator 2) of joints. Concordance correlation coefficients between MRI and histopathology were better for defect depth (Evaluator 1: 0.68-0.84; Evaluator 2: 0.76-0.83) compared to width (Evaluator 1: 0.30-0.54; Evaluator 2: 0.48-0.68). However, MRI overestimated defect depth (histopathology: 1.65 +/- 0.94 mm; Evaluator 1, range of means: 2.07-2.38 mm; Evaluator 2, range of means: 2-2.2 mm) and width (histopathology: 6.98 +/- 1.32 mm; Evaluator 1, range of means: 8.33-8.8 mm; Evaluator 2, range of means: 6.64-7.16 mm). Using the paired t-test, the mean T2 relaxation time of cartilage defects was significantly greater than the mean T2 relaxation time of the adjacent normal cartilage for both evaluators ($p<0.0001$). 3T MRI accurately identified canine articular cartilage defects in this cadaveric study. Additional studies are necessary to evaluate the ability of MRI to accurately identify canine articular cartilage defects *in vivo*.

Introduction

Articular cartilage injury is a component of many canine orthopedic diseases, such as cranial cruciate ligament rupture, hip dysplasia, elbow dysplasia, septic arthritis, immune-mediated arthritis, osteochondrosis, meniscal injury, and intra-articular fracture. While treatment is often aimed at addressing the underlying cause in order to slow disease progression, articular cartilage injuries often worsen with time, contribute to future lameness, and lead to a diminished quality of life through the development and progression of osteoarthritis (OA).[1]

A fundamental concept of treating osteoarthritis is that treatment strategies are often more effective when applied early in the disease process, prior to development of morphologic change.[2] However, early detection and treatment of OA and articular cartilage injuries in dogs has proven to be a challenge. Radiography is commonly used to diagnose osteoarthritis, but most radiographic signs of osteoarthritis are associated with chronic disease.[1] It has been estimated that 40% of periarticular bone must be lost before it becomes evident radiographically.[3] Moreover, radiographs do not accurately assess the radiolucent articular cartilage and are an inaccurate indicator of the structural integrity of articular cartilage.[4] While arthroscopy provides a thorough visual evaluation of the articular cartilage surface, it requires general anesthesia and surgery.

A wide variety of novel treatment options for focal articular cartilage injury have been developed for use in humans, including: pharmacologic agents, osteotomies to redistribute loads to unaffected areas of the joint, autologous chondrocyte implantation, and osteochondral transplantation. Additionally, orthobiologics such as platelet rich plasma, stem cell therapy, and autologous conditioned sera are of interest. Several of these techniques have been evaluated in

62 dog models of osteoarthritis and are currently in clinical use.[5-14] A non-invasive imaging
63 modality that provides adequate resolution to identify articular cartilage defects is necessary to
64 evaluate the efficacy of these novel treatments. MRI has become the imaging modality of choice
65 for evaluating articular cartilage in humans because of its non-invasive nature and the ability to
66 directly image both articular cartilage and subchondral bone.[4, 15] To date, a modest number of
67 reports have described the use of MRI for evaluation of articular cartilage in dogs. The majority
68 of these reports use low field magnets and subjective image evaluation.[16-19]

69 Recent work in humans has led to the development of quantitative measures of cartilage
70 degradation using MRI.[15, 20-22] T2 mapping is an MRI procedure developed to assess collagen
71 structure of articular cartilage. T2 maps display the T2 relaxation time of tissue on a voxel by
72 voxel basis.[23] A recent study evaluated T2 mapping in the normal canine elbow and found a
73 mean T2 relaxation time of the articular cartilage of 56 ± 8 milliseconds.[24] Fast-low angle shot
74 (FLASH) is an isotropic three-dimensional T1-weighted, fat-suppressed, gradient-recalled echo
75 sequence with radiofrequency (RF) spoiling that has been used to evaluate articular cartilage
76 surface defects in humans.[20, 25] Proton density-weighted (PD) imaging is effective for
77 morphologic evaluation of articular cartilage injury in humans and is effective in the evaluation
78 of other soft tissue structures, such as ligaments and menisci.[15, 26] T1 weighted imaging has been
79 shown to be useful in evaluating subchondral bone.[27] While T2 mapping, FLASH, PD-weighted,
80 and T1- weighted imaging have been used successfully to characterize human articular cartilage,
81 their usefulness in the evaluation of canine articular cartilage remains unclear due to low signal
82 to noise ratio and poor in plane resolution in prior studies.

83 The purpose of this study was to determine if small field of view 3.0 tesla (3T) MRI was capable
84 of identifying experimentally induced articular cartilage defects in canine cadaveric stifles. We

hypothesized that quantitative evaluation of T2 mapping, proton density-weighted, FLASH, and T1-weighted sequences using 3T small field of view (7.0 cm) would accurately identify the location, depth, and diameter of articular cartilage defects. Furthermore, we hypothesized that T2 relaxation times of experimentally induced articular cartilage defects would be significantly different than that of adjacent cartilage.

Methods

Twenty-one pairs of canine pelvic limbs (42 total limbs) were obtained from healthy dogs (30-50 kg) euthanized for reasons unrelated to this study. As the study involved cadaveric tissues, Institutional Animal Care and Use Committee approval was not required. Limbs were disarticulated at the hip joint and frozen at -20°C until the day prior to evaluation. Limbs were thawed in a water bath at room temperature. Following thawing, survey radiographs and MRI (FLASH, T1-weighted, PD-weighted, and T2 mapping) were used to screen for pre-existing osteoarthritis or other pathology in the stifle joint such as cranial cruciate ligament rupture or patellar luxation. The MRI protocol used to screen for pathology was identical to the protocol used following defect creation (Table 1). Stifles with pre-existing pathology on radiographs or MRI were excluded from the study by consensus evaluation between the resident investigator, board-certified radiologist, and board-certified orthopedic surgeon. Limbs were assigned to six experimental groups or a sham-operated control group, resulting in 6 limbs per treatment group (Table 2). Defects in the experimental groups were created in the medial or lateral femoral condyle at a depth of 0.5 mm, 1.0 mm, or 3.0 mm. Femoral condyles were selected because these are locations within the stifle joint in which early articular cartilage lesions are commonly

encountered in clinical patients[28] and the femoral condyles are accessible with minimal disruption of surrounding tissues. Multiple defect depths were selected to assess the ability to identify defects of varying clinical severity using the described imaging protocol and 3T small field of view MRI. The 0.5 mm defect simulated a partial thickness articular cartilage lesion. The 1.0 mm defect simulated an articular cartilage lesion that extended through the subchondral bone plate. The 3.0 mm defect simulated an articular cartilage lesion that extended through the subchondral bone plate and into the adjacent cancellous bone.

For all limbs, articular cartilage defects were created by a board-certified small animal surgeon. Stifles were approached via lateral parapatellar arthrotomy. A 2 cm segment of the tendon of origin of the long digital extensor muscle was excised from each limb. Resection of this tendon was required in order to position the custom designed drill bit and depth collar perpendicular to the surface of the lateral femoral condyle. Tendon resection was performed in all limbs to avoid bias during image evaluation. Under constant saline lavage, a cartilage defect was created in the medial or lateral femoral condyle at one of three depths (0.5 mm, 1.0 mm, or 3.0 mm) using a custom designed drill bit and depth limiter (Figure 1). The drill bit used was a 7.94 mm coarse grit tungsten carbide bur (Foredom Electric Co, Bethel, CT). A custom-designed depth collar and hand piece were designed (Precision Tool & Engineering, Gainesville, FL) to control drill bit penetration and to minimize the pressure applied to the healthy cartilage adjacent to each defect bed. The collar on the drill bit was also designed to orient the drill bit perpendicular to the cartilage surface during defect creation. Limbs assigned to the sham-operated control group underwent the same surgical protocol as the experimental group without creation of articular cartilage defects. Next, limbs were fully submerged in an isotonic saline bath. The stifle joint was lavaged with saline during flexion and extension of the stifle joint to eliminate gas from the

joint space. The joint capsule, subcutaneous tissues, and skin were closed using simple continuous suture patterns while submerged in the saline bath to ensure gas did not re-enter the joint during closure.

Following defect creation, MRI was performed using a 3T system (Siemens Magnetom Verio, Malvern, PA). A 15 channel transmit/receive knee coil was used (Quality Electrodynamics, Cleveland, OH). Limbs were positioned within the coil as if the patient was in dorsal recumbence with the hip joints extended. FLASH, T1-weighted, PD-weighted images, and T2 maps of each stifle joint were acquired in dorsal and sagittal planes (Table 1 and Figure 2). This resulted in a total of 168 sequences evaluated (42 limbs, 4 sequences per limb). Dorsal and sagittal plane images were selected because they have been shown to be more useful than transverse plane images in the canine stifle joint.[29] T2 maps were generated using *syngo* MapIt (Siemens Healthcare, Malvern, PA). Total scan times for the cadaveric imaging study was 240 minutes.

MRI images were evaluated independently by two board-certified radiologists blinded to treatment group. Dorsal and sagittal plane images for each sequence were randomized into 168 image pairs. Dorsal and sagittal plane images for each sequence (T1-weighted, PD-weighted, T2 maps, and FLASH) were evaluated together, but each sequence was evaluated independently from the other sequences. This method was selected to mimic the process investigators use to evaluate client owned animals. Because the two planes were evaluated concurrently, no attempts were made to determine whether defects were easier to identify on dorsal or sagittal plane images. Evaluators assessed the medial and lateral femoral condyles for the presence or absence of a defect. If present, the evaluators measured the defect width and depth at the deepest point in the center of the defect. Lesion depth was measured on dorsal plane images at the deepest point

153 in the central third of the lesion with respect to mediolateral direction. Lesion diameter was
154 measured in a craniocaudal direction on sagittal plane images. T2 relaxation times were
155 measured on the T2 maps within the center of the defect at the level of the articular cartilage. T2
156 relaxation times of the adjacent articular cartilage on the caudal aspect of the defect were also
157 measured in all dogs with articular cartilage defects. If no defect was identified, T2 relaxation
158 times were not measured. All measurements were made on a dedicated Siemens workstation
159 (Siemens Healthcare, Malvern, PA).

160 Following MRI, the soft tissues were dissected from the femur, and the femur was disarticulated
161 from the tibia. A band saw was used to obtain approximately 3 mm thick portions of the medial
162 and lateral femoral condyles. For condyles containing a defect, these gross sections were cut to
163 ensure the center of the defect was included in each tissue block. For condyles without a defect,
164 the 3 mm thick tissue block was obtained from the center of the condyle in a mediolateral
165 direction (sagittal plane). Femoral condyles were fixed in 10% buffered formalin and then
166 decalcified using a 25% buffered formic acid solution (25 ml formic acid, 100 g sodium citrate,
167 and 75 ml distilled water). Each condyle was washed in tap water and stored in 70% ethyl
168 alcohol. Additional dehydration was performed using 70%, 85%, 95%, and 100% (twice) ethyl
169 alcohol followed by xylene (three times). Condyles were paraffin embedded, and hematoxylin
170 and eosin slides of each condyle were prepared for histopathologic measurement. Slides were
171 examined by an experienced veterinary orthopedic pathologist blinded to treatment group and
172 MRI findings. Photomicrographs were taken of each section using digital photography at 4 x
173 magnification. Each image contained a 1 mm standard. Images were imported into a
174 commercially available software program (eFilm workstation 3.3, Sound-Eklin, Carlsbad, CA),
175 and a linear calibration tool was used to calibrate each image to the 1 mm standard. Each

condyle was evaluated for presence or absence of a defect. Maximum defect width and depth were recorded for each condyle.

Descriptive statistics were generated for histopathologic and MRI measurements using Microsoft Excel version 10.0 (Microsoft Corporation, Redmond, WA). Concordance correlation coefficients were generated and paired t-tests were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Accuracy of defect identification for MRI was determined by comparing image evaluator defect location to histopathologic measurements, with histology serving as the gold standard. For each sequence and investigator, MRI and histopathologic measurements of defect width and depth were compared using concordance correlation coefficients. Concordance correlation coefficients were also used to evaluate agreement of MRI measurements between evaluators. Defects that were incorrectly identified as controls (no defect) were excluded from calculation of concordance correlation coefficients comparing MRI and histopathology measurements and observer agreement. Including a defect measurement of 0 mm for defects that were incorrectly identified as controls would have artificially affected these calculations. For analysis of T2 relaxation times, the mean T2 relaxation times (+/- standard deviation) were determined for the articular cartilage defects and for the adjacent normal articular cartilage. T2 relaxation times within the articular cartilage defect were compared to the adjacent normal articular cartilage using the paired t-test.

Results

Evaluator 1 correctly determined defect status in 98.8% (166/168) of the evaluated sequences, while Evaluator 2 correctly determined defect status in 98.2% (165/168) of the evaluated

198 sequences (Table 3, Figure 3). Evaluator 1 correctly determined defect status for all T1 and PD-
199 weighted images. Evaluator 1 incorrectly classified a defect in the 0.5 mm medial condyle group
200 as a control on FLASH images. One defect in the 1.0 mm medial condyle group was correctly
201 identified as a defect but was incorrectly localized to the lateral femoral condyle on the T2 map.
202 This was attributed to human error during data recording. Measurements for this defect were
203 included in the calculation of concordance correlation coefficients and mean defect
204 measurements. Evaluator 2 correctly determined defect status for all FLASH, PD-weighted, and
205 T2 maps. Evaluator 2 incorrectly classified 3 defects in the 0.5 mm groups (2 lateral condyle
206 and 1 medial condyle) as controls on T1-weighted images (Figure 4). Evaluator 2 was unable to
207 measure the diameter of one defect in the 1.0 mm lateral femoral condyle group on FLASH
208 images, likely due to susceptibility artifact associated with defect creation.

209 Mean +/- standard deviation defect depth and defect width, as determined by histopathology and
210 MRI, are reported in Table 4. Defect depth determined by MRI was greater than defect depth
211 determined using histopathology for all sequences, regardless of the evaluators. Mean defect
212 width determined by Evaluator 2 was slightly smaller than the mean defect width measured on
213 histopathology for FLASH and T1-weighted images. The mean defect width measured using
214 MRI was greater than the mean defect width on histopathology for all sequences for Evaluator 1
215 and for PD-weighted images and T2 maps for Evaluator 2.

216 Concordance correlation coefficients comparing MRI and histopathology measurements for each
217 evaluator are shown in Table 5. For both evaluators, there was stronger correlation (i.e. the
218 correlation coefficient was closer to the value of "1", which represents complete correlation)
219 between MRI and histopathology measurements for defect depth as compared to defect width.
220 Differences between concordance correlation coefficients for defect depth and width were

greater for Evaluator 1 than Evaluator 2. When compared to histology, correlation of MRI was stronger for defect depth measurements as compared to defect width measurements for all sequences evaluated. However, no sequence exhibited markedly superior correlation with histopathology measurements for either evaluator when considering both lesion depth and width.

Concordance correlation coefficients comparing MRI defect measurements between evaluators are shown in Table 6. Correlation between evaluators' MRI defect measurements was greater for defect depth than width. Correlation between evaluators' MRI defect depth measurements was similar for all sequences. Correlation between evaluators' MRI defect width measurements was greatest for T2 maps. Correlation between evaluators' MRI defect width measurements was poor for the remaining sequences. Furthermore, while the correlation between defect width measurements was lower than for defect depth measurements, of the sequences evaluated in this study T2 mapping was the most precise method to estimate defect width (i.e. width measurements were most similar between the two investigators).

The T2 relaxation times for articular cartilage defects was 690 ± 379 ms for Evaluator 1 and 336 ± 190 ms for Evaluator 2, whereas the T2 relaxation time for adjacent normal cartilage was 53.1 ± 9.4 ms for Evaluator 1 and 46.9 ± 15.7 ms for Evaluator 2 (Table 7). Using the paired t-test, the mean T2 relaxation time of cartilage defects was significantly greater than the mean T2 relaxation time of the adjacent normal cartilage for both evaluators ($p<0.0001$).

Discussion

Experimentally induced articular cartilage defects of varying depths were correctly identified with FLASH, T1-weighted, PD-weighted, and T2 maps using 3.0 tesla MRI with a 7.0 cm field of view. There was superior correlation between MRI and histopathology measurements for

243 defect depth compared to defect width for both evaluators. Overall, MRI tended to overestimate
244 both defect depth and width measurements. There was superior inter-observer agreement for
245 defect depth measurements compared to defect width measurements. No sequence was
246 consistently more accurate for the identification and measurement of cartilage defect depth
247 between evaluators, whereas there was improved correlation between evaluators' defect width
248 measurements using T2 maps when compared to the other sequences evaluated. To the authors'
249 knowledge, this is the first study that has evaluated the use of MRI for identifying articular
250 cartilage defects in the canine stifle joint using high field strength, small field of view MRI.
251 While any of the sequences evaluated in this study appear to be sufficient for determining lesion
252 depth in canine articular cartilage, T2 maps should be considered for determining the lesion
253 width due to the more precise nature of T2 mapping of width measurements documented
254 between the two investigators in this study.

255 MRI of articular cartilage is more challenging in dogs than humans because canine articular
256 cartilage is thinner. In one study, the articular cartilage and subchondral bone thickness
257 measured 2411 ± 30 μm on the proximal medial femoral condyle in humans compared to 771 ±
258 30 μm in dogs and 2523 ± 30 μm on the distal medial femoral condyle in humans compared to
259 731 ± 30 μm in dogs.[30] This makes it more difficult to design MRI sequences with adequate
260 spatial resolution to evaluate canine articular cartilage with a scan time that is clinically relevant.
261 The total scan time for the protocol used in this study was approximately 240 minutes. This scan
262 time is likely too long to be widely applicable to anesthetized canine veterinary patients.
263 However, due to the fact that this was a cadaveric study, an optimal imaging protocol was used.
264 It is likely that our ideal protocol will need to be modified for clinical patients to acquire a
265 quality MRI exam in an acceptable amount of time. Optimization of MRI sequences and scan

times was not the objective of this study and should be the subject of future work. Furthermore, advancements in MRI and coil technology will likely reduce scan times while maintaining or increasing spatial resolution.

Articular cartilage defect status was correctly identified by both evaluators in greater than 98% of the MRI sequences evaluated in this study (Table 3). The defect that was incorrectly localized to the lateral femoral condyle on the T2 map by Evaluator 1 was likely due to evaluator data recording error rather than an inability to correctly identify the defect. The remaining 4 defects incorrectly identified as controls were in the 0.5 mm defect groups and were more subtle defects. Of these, 3 defects were not identified by Evaluator 2 on T1-weighted images. It was expected that defect identification would be more challenging on T1-weighted images because of low contrast between articular cartilage and joint fluid. The remaining incorrect defect identification was a 0.5 mm medial condyle defect identified as a control by Evaluator 1 on FLASH (Figure 4).

Correlation of MRI measurements with histopathology defect measurements was variable. Some of this variability may be related to differences in defect measurement location between the MRI and histopathology. On the MRI, defects were measured at the deepest point in the central third of the defect with respect to the mediolateral direction. Histopathology sections were obtained from 3 mm thick tissue blocks visually cut through the central third of the defect. However, histopathology sections are 5 μm thick. Several sections were made from each defect and included for histopathology evaluation, but it is possible that the defect measurement location on MRI did not precisely match the histopathology defect measurement location. Additionally, given the 2 mm slice thickness, partial volume averaging could contribute to the discrepancies between MRI and histopathology measurements. Defect depth measurements using MRI were more accurate than defect width measurements. While the base of each defect was relatively

289 uniform, defect edges were often irregular, making them more difficult for evaluators to identify.
290 Additionally, despite copious lavage during drilling, artifacts created by debris associated with
291 defect creation made identifying margins of the defects difficult in several limbs (Figure 5).
292 Defect depth measurement was likely more accurate because of a clearer distinction between the
293 defect and subchondral bone plate or underlying cancellous bone bed. Because the femoral
294 condyle is a rounded surface, evaluators had to estimate where the articular cartilage surface
295 would have been located if a defect was not present on both MRI and histopathology. This also
296 likely contributed to the difference between MRI and histopathology measurements. Correlation
297 between MRI and histopathology for defect depth was inferior for T2 maps than other sequences.
298 This could have been due to lower spatial resolution of the T2 maps. The matrix size for the T2
299 maps was 192 x 192 compared to 384 x 384 for the other sequences with the same field of view
300 (7 cm) and slice thickness (2.0 mm).

301 Correlation of defect measurements between evaluators varied between defect depth and width.
302 There was better agreement between evaluators' measurements for defect depth as compared to
303 defect width. This was likely because many of the defect margins were not sharply demarcated
304 resulting in differences in defect margin identification between evaluators as described above.
305 Additionally, although the defects and joints were flushed with saline following defect creation,
306 several of the limbs had susceptibility artifacts from a small amount of residual debris that likely
307 contributed to differences in defect margin identification (Figure 5). These factors are less likely
308 to play a significant role in defect depth measurements because of the sharp demarcation
309 between cartilage defects and subchondral or cancellous bone.

310 As hypothesized, the T2 relaxation times within the articular cartilage defects were significantly
311 different than the T2 relaxation times of the adjacent normal articular cartilage. The T2

relaxation time within the articular cartilage defect was expected to be similar to that of joint fluid. There was a wide range in the T2 relaxation times of the articular cartilage defects for both evaluators. This was likely because the articular cartilage defects were filled with a mix of saline, joint fluid, and small amounts of residual debris secondary to defect creation. The T2 relaxation time of synovial fluid has been reported to be 767 ± 48.8 msec at 3T[31], which is slightly higher than the T2 relaxation times of the articular cartilage defects in our study. Although all of the joints were thoroughly lavaged with saline prior to closure, based on the non-uniform appearance of the defects on MRI in some of the limbs, it is likely a very small amount of debris remained. The T2 relaxation times were determined by measurement of a single pixel. Because of the suspected heterogeneity of the fluid within the joint adjacent to the defects, unless evaluators measured the same pixel, there was likely a large amount of variability between measurements.

As with all cadaveric studies, our study had several limitations. Evaluating articular cartilage is most useful in patients with osteoarthritis. Our study design evaluated experimentally-induced articular cartilage defects in cadaveric stifles, which cannot completely simulate naturally occurring osteoarthritis. However, using our model, defects from all groups were correctly identified using the selected MRI sequences. This suggests that the MRI sequences utilized in our study may be clinically applicable if the scan time can be significantly reduced. However, additional studies evaluating articular cartilage lesions *in vivo* using experimentally induced osteoarthritis or clinical patients with osteoarthritis are needed to further evaluate the clinical relevance of these sequences. Additionally, because the defects were experimentally induced in canine cadavers, there were occasional susceptibility artifacts present due to a small amount of intra-articular gas and debris within the joint, some of which were adjacent to the cartilage

defects. Susceptibility artifacts are more common with gradient recalled echo sequences, such as FLASH. In some cases, this may have made defect identification easier but may have hindered accurate defect measurement, particularly defect width. Finally, because the defects were created on the rounded surface of the femoral condyle, the defect depth was less uniform than if defects were created on a flat surface. However, the femoral condyle was selected because this is a common location for canine patients to develop articular cartilage lesions and it was readily amenable to perpendicular drilling via lateral parapatellar arthrotomy.

The limbs used in this study had been previously frozen, and several studies have indicated tissue properties measured using MRI can be affected by prior freezing[32, 33]. However, a previous study evaluating the effects of freezing and refrigeration on the electromechanical and biomechanical properties of articular cartilage suggested that freezing of articular cartilage at -20ºC does not significantly affect these properties. [34] In addition, a previous study evaluating human articular cartilage reported similar T2 relaxations times for normal, non-frozen articular cartilage and previously frozen cartilage[35, 36]. The T2 relaxation times for the normal articular cartilage adjacent to the defects was similar to a study that reported the T2 relaxation time of the articular cartilage in the normal canine elbow joint of clinical patients to be 56 ± 8 ms[24]. Although prior freezing of the cadaver limbs could have affected the T2 relaxation times of the articular cartilage, we expect that this effect was minimal.

Finally, histology was used as the gold standard for determining defect dimensions in this study. Tissue processing for histology has been shown to decrease the dimensions of the liver and kidney[37]. Based on the existing literature, the effect, if any, of tissue processing on the dimensions of articular cartilage and bone are unclear. Several studies have used histologic measurements as gold standards in evaluation of articular cartilage[38-44]. It is possible that

shrinkage of the articular cartilage during tissue processing could have contributed to the overestimation of articular cartilage defect dimensions on MRI, however this has not been the authors' experience.

In conclusion, the MRI sequences evaluated in this study accurately identified experimentally induced articular cartilage defects in the canine stifle. There was superior correlation between MRI and histopathology measurements for defect depth as compared to width. There was superior inter-observer agreement for defect depth measurements. In general, measuring articular cartilage defects in our model of cartilage injury using MRI tended to overestimate defect size. Future studies should be considered to evaluate these sequences in canine patients with naturally occurring osteoarthritis.

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